STRATEGIES FOR ELIMINATION OF *SALMONELLA* TYPHIMURIUM

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Four different strategies were employed to try to eliminate *Salmonella* typhimurium from infected herds or pigs. Only strategic removal of pigs prior to entering infected sections of the herd was found to be beneficial. Strategies involving efforts to eliminate salmonella from infected pigs or herds by medication were found to be inefficient, and a vaccination strategy using a killed salmonella vaccine did not reduce subclinical infection.

MATERIALS AND METHODS

Bacteriology and serology: Bacteriological examinations were performed using standard microbiological methods (nonselective pre-enrichment followed by selective enrichment and serotyping). A pen sample is by definition 25 g of faecal material pooled from 5 different parts of the pen. Blood samples were analysed using the Danish Salmonella-mix-ELISA, using a cut-off value of 10 OD% to discriminate between positive and negative samples.

Method 1. Experimental design: Three herds (A, B and C) with verified, persistent high levels of infection with *S.* typhimurium were selected. All herds used heat treated compound feed. No medical routine treatments were used before or after removal of pigs.

Pen samples were collected from nurseries, growers units and finishers units in all herds to investigate the localization of salmonella in the herds.

Herd A: At birth a total of 92 pigs from 23 sows farrowing in one week were randomly selected, equally distributed to sex and litter. At weaning one male and one female pig in each litter (total 46 pigs) were removed to 9 pens in isolated facilities outside the farm. Forty-four siblings were kept on farm A as controls following the usual management procedure on the farm. Two pigs died before weaning with no signs of salmonellosis. All removed pigs were bled at weaning (21 days), at 10 and 16 weeks of age, and at the time of slaughter (20 weeks of age), and all controls at weaning, at 10 and 16 weeks of age. Rectal swabs from all removed and control pigs were collected at weaning and pooled in 12 groups. Pen samples from the external finishing unit were collected at 10 and 16 weeks of age and at slaughter (20 weeks of age) and from the control group at 10 and 16 weeks of age.

Herd B: Approximately 700 pigs were removed from the nurseries at 10 weeks to a finishing unit outside the farm over a period of 6 months. The external finishing unit was cleaned and disinfected prior to the first arrival of pigs, but not cleaned thereafter. The external unit was continuously managed and no effort was made to avoid faecal contact between pens. Approximately 800 finishers concurrently produced in a continuously managed finishing unit within herd B served as controls. At slaughter, 60 blood samples from removed pigs and 88 blood samples from control pigs were collected. 5 g of caecal content from 10 control pigs and 10 pigs in the external finishing unit were bacteriologically examined for salmonella.

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Herd C: A new finishing unit was built. The formerly infected growers unit was emptied completely, cleaned and disinfected. Hereafter the growers unit was gradually filled again. At 30 kg bodyweight 98 pigs were removed from the growers unit to 4 pens in the new finishing unit. Measures were taken to avoid transfer of infectious material from the old finishing unit (separate boots and tools for each unit). In the continuously managed system 175 finishers served as controls. At slaughter 30 blood samples from the new finishing unit and 30 blood samples from the old finishing unit were collected. 5 g of caecal content from 10 pigs in the new finishing unit were bacteriologically examined for salmonella. No pigs from the control group were bacteriologically examined.

Method 2: Thirty gilts from a herd known from previous microbiological tests to be heavily infected with *S.* typhimurium were medicated with enrofloxacin (Baytril^{R} inj. 100 mg/ml) 5 mg/kg bw. pr day for 4 days before removal to cleaned and disinfected facilities on another location, where medication continued for 7 days as a topdressing on feed (Baytril premix 2.5 %) at a dosage of 5 mg/kg. Faecal samples from 17 gilts were collected 57 days after transport.

Method 3: Herd D and E were both 240 sow herds with continuous production in farrowing sections, all in-all out in the weaner sections, and continuous production in grower section. Herd D produced all finishers in an all in-all out facility on a nearby farm. Herd E produced finishers in a continuously managed finisher section on the farm. *S.* typhimurium DT 12 was isolated from pen samples in grower sections and finisher sections, but not from the weaner sections, in both farms. Both herds used feed processed in accordance with the Danish *Salmonella* program. Both herds followed a rodent control program.

Both herds followed the same intervention program, except for finishers. All growers, finishers (herd E) and gilts under 10 month of age were removed from the herd. The empty sections were thoroughly cleaned using high pressure hosing. The sections were fumigated with formaldehyde, and residual slurry in the pits were disinfected with slaked lime (50 kg lime/m^3). Part of the farrowing section was sealed off from the rest of the section with plastic walls and high pressure hosed and fumigated with formaldehyde. During the medication period, the entire farrowing section was cleaned by moving the walls to a new part each week. Weaning sections were cleaned and fumigated with formaldehyde, and the slurry pits were emptied and disinfected with lime. In the dry sows sections and farrowing sections all faecal material was removed daily, and the defecation area was disinfected with an oxidizing disinfectant. Sows, boars and weaners were medicated with enrofloxacin premix 2.5 % (Baytril^{R}) used as a topdressing for sows and boars and as medicated feed for weaners. The treatment period was 2 x 14 days with a 1 week break. Dosage was 5 mg/kg bw for all groups. All sucklings were treated three times during the period with enrofloxacin inj. (Baytril), dosage 5 mg/kg bw. on day 1, 3 and 5 of the medication period. During the medication period separate boots and tools were used for all sections. After the medication period the grower section in herd D was gradually restocked. The finisher sections on the nearby farm was emptied one by one, and cleaned and disinfected following the same procedures as described earlier. Boots and clothes were changed when going from one section to another. In herd E the grower section was rebuilt into a finisher section. Finisher sections were gradually restocked. After the treatment period, both herds were examined bacteriologically.

Method 4: 18 salmonella free pigs, 11-12 weeks of age, were randomly allocated to 2 groups of 9 pigs. Each group was situated in strictly separated units. Both groups were vaccinated subcutaneously on day 34 and 21 days before challenge, dose 2 ml. Vaccine for group 1 was a formalin-inactivated whole cell vaccine containing 10^9 *S.* dublin, 10^9 *S.* typhimurium and 10 strains of bovine *E.* coli (Bovivac, Hoechst Animal Health) pr ml. Vaccine for group 2 was a formalin-inactivated whole cell vaccine containing 12 strains of porcine *E.* coli isolates (Porcovac^R plus vet, Hoechst) pr ml. All pigs were challenged orally day 0 with $5*10^5$ CFU of *S.* typhimurium DT 12.

After challenge pigs were examined clinically daily for 6 days. Individual faecal samples were collected every 2-3 days after challenge for 3 weeks. Selected organs were collected after sacrifice day 48-50 post challenge.

RESULTS

Method 1: Salmonella was not isolated from nurseries in any of the herds, but *S.* typhimurium was isolated from growers or finishers units in all herds prior to the trial. All microbiological and serological samples from pigs removed from herd A, B and C were negative. In herd A, *S.* typhimurium was isolated from all pen samples in the control group at 16 weeks of age, and 39 out of 40 control pigs were serologically positive. In herd B ten out of 88 control pigs were seropositive at slaughter, and *S.* typhimurium was isolated from one caecal sample. In herd C 16 out of 30 controls were seropositive.

Method 2: S. typhimurium was isolated from 2 out of 17 gilts after the medication period.

Method 3. In herd D, grower section was examined bacteriologically twice after medication. *S.* 4.12 b-.-. was isolated from 1 pen sample. One year later the herd was re-examined bacteriologically in all sections with negative result. In the external finishing section, *S.* typhimurium DT 12 was isolated in several pens in the finishing sections after the treatment period. In herd E, *S.* typhimurium DT 12 was isolated in several pens in the finishing section after the treatment period.

Method 4. A modest but significant reduction in clinical impact was observed in the Bovivac-group. Most pigs excreted 10^{12} CFU *S.* typhimurium at least once after challenge. There was no difference in faecal shedding and recovery of challenge strains in organs.

DISCUSSION AND CONCLUSION

Method 1: It cannot be determined whether the apparent lack of transfer of infection from the sow to the removed pigs is due to absence of salmonella infection in the sows, or to low susceptibility of the piglets, perhaps as a result of antibodies in colostrum or milk. It seems reasonable to assume, that the control pigs raised within the infected units were infected from either residual infection in the pens or from older pigs in the unit.

Method 2 and 3: Medication of highly infected pigs before and after introduction into the herd will not eliminate salmonella. Medication of all animals in 2 herds did not eliminate the infection from the herd. Not only do we not find medication effective, but there is a risk of development of antibiotic resistance in salmonella. It cannot be stated whether the failure was due to residual infection in the environment or to infection still remaining in the breeding stock.